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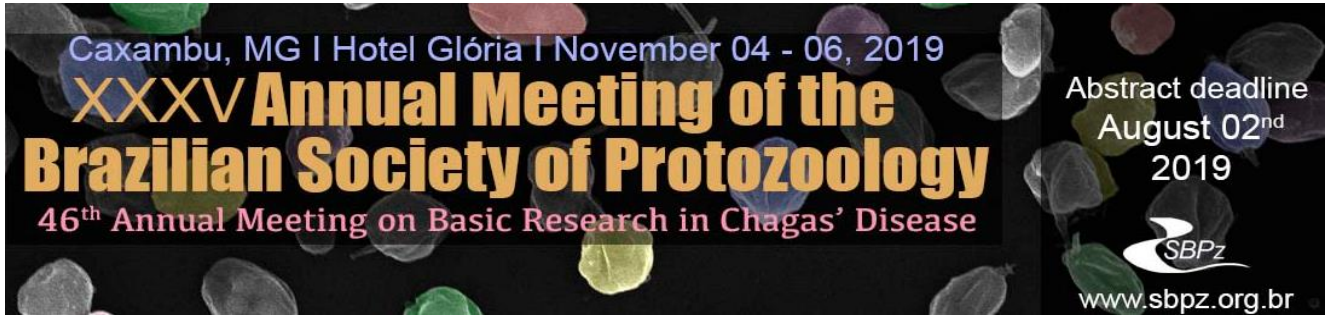

TB14 - ***TRYPANOSOMA CRUZI* INOSITOL PHOSPHORYLCERAMIDE SYNTHASE AS A POTENTIAL DRUG TARGET FOR CHAGAS DISEASE**

ESTEVEZ CASTRO, C.F.*[1]; FERREIRA CHAME, D.[1]; GRECO, R.S.[1]; FERNANDES, H.B.[1]; CARDOSO, M.S.[1]; DENNY, P.W.[2]; TEIXEIRA, S.M.R.[1]

1.UFMG, BELO HORIZONTE, MG, BRASIL; 2.DURHAM UNIVERSITY, DURHAM, UK.

e-mail:carlosestevez45@gmail.com

Sphingolipids (SLs) are an essential part of all eukaryotic cellular membranes. Inositol phosphorylceramide (IPC) is an abundant SL present in numerous protozoa but absent in mammals. IPC synthase is an integral membrane protein that is conserved between kinetoplastids such as *Trypanosoma cruzi* and *Leishmania major* and constitutively expressed throughout their cell cycle. Thus, IPC synthase constitutes an ideal target for the development of new treatments for Chagas disease, an illness caused by *T. cruzi* infection, which still lacks an effective treatment. Because inhibitory drugs of the *L. major* IPC synthase were recently identified, the objective of this study is to evaluate their effect on the *T. cruzi* enzyme, named TcIPCS, using two types of genetically modified parasites: *TcIPCS* knockout mutants generated with the CRISPR-Cas9 technology and parasites over-expressing the *TcIPCS* gene. To obtain the *TcIPCS* knockouts, epimastigotes were simultaneously transfected with in vitro synthesized small guide RNAs complexed with recombinant *Staphylococcus aureus* Cas9 and a DNA construct containing the neomycin resistance gene flanked by *TcIPCS* neighboring regions. After neomycin selection, cloning and genotyping, parasites with both alleles disrupted were obtained. To generate *TcIPCS* over-expressing parasites, the pROCKHygro expression vector containing the *TcIPCS* coding region with an HA tag was transfected into epimastigotes. After hygromycin selection, cloned cell lines over-expressing the tagged enzyme were characterized by qPCR and immunofluorescence analysis. *TcIPCS* KO parasites showed a subtle growth defect and decreased metacyclogenesis. Also, *TcIPCS* deletion resulted in decreased in vitro infection capacity, intracellular replication and trypomastigote release. In vitro characterization of parasites overexpressing *TcIPCS* as well as tests using compounds with TcIPCS inhibitory potential on both *TcIPCS* mutant cell lines are underway. **Supported by:**CNPq; FAPEMIG; Global Challenges Research Fund; Global Network for Neglected Tropical Diseases. **Keywords:** Trypanosoma cruzi; inositol phosphorylceramide synthase; crispr-cas9